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Simple and Versatile Method for Tagging Phenyldiazirine Photophores

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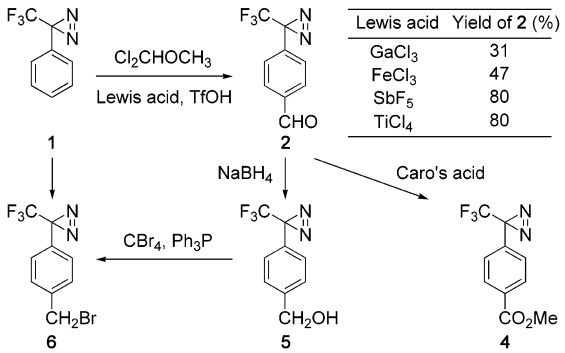
Efficient access to useful diazirine photophores has been developed. Although 3-phenyl-3-trifluoromethyl diazirine derivatives satisfy most of the chemical criteria required for photoaffinity labeling,[1] the limited availability of conveniently modified derivatives represents a major drawback. Alkoxy-substituted phenyldiazirines are currently the only useful synthetic units for evading the repetition of time-consuming multistep diazirine synthesis.[2,3] 3-Phenyl-3-trifluoromethyl-3*H*-diazirine (**1**), the simplest analog, can also be prepared easily on a large scale. However, the use of this building block has been hampered by the low reactivity of its simple benzene ring[3] and the instability of the diazirine ring to the acidic conditions of conventional Friedel−Crafts reactions.[4,5] The carboxylation of **1**, mediated by a toxic thallium salt, has been reported,[6] but only moderate yields of an isomeric mixture were obtained after 24 h of reaction. A breakthrough in the direct modification of **1** would provide access to a range of valuable photoaffinity probes. Here we report the efficient preparation of synthetically useful aldehyde **2** from **1**, which has enabled a variety of applications, including the catalytic asymmetric synthesis of the photoreactive phenylalanine analog L-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-phenylalanine (TmdPhe, **3**). The straightforward synthesis of **3** provides sufficient TmdPhe for convenient automated peptide synthesis. Thus, a TmdPhe-containing calmodulin-binding peptide (CaMBP) was easily obtained, which specifically photolabeled calmodulin (CaM).

Although C−C bond formation on the benzene ring of **1** is crucial for effective derivatization, only a limited number of reactions can be used for this purpose because of the rather labile diazirine ring and the low reactivity of the benzene ring. We previously found that the Friedel−Crafts reaction of **1** with $GaCl_3$ and dichloromethyl methyl ether (a suspected carcinogen) produced the desired aldehyde **2** in the presence of TFA, albeit in low yield.[5] Under these conditions, the diazirine group was stable, whereas the aldehyde source, dichloromethyl methyl ether, rapidly decomposed in the acidic medium, leaving large amounts of unreacted **1**. Dichloromethyl methyl ether was reported to be stable in trifluoromethanesulfonic acid (TfOH) and can be used successfully for Friedel−Crafts acylation of inert benzene rings.[7] Indeed, we find that if we use SbF_5 in TfOH at 0 °C, formylation of **1** proceeds in 80% yield to give the desired aldehyde **2**, a compound that was previously obtained only in small quantities after many synthetic steps.[5] Triflates of Ag, Pr, Y, Yb, Gd, or Sm in TfOH were ineffective, whereas $GaCl_3$ and $FeCl_3$ afforded **2** in moderate yields (31% and 47%, respectively), and $TiCl_4$ gave a sufficient yield. The results are summarized in Scheme 1.

Scheme 1. Synthesis of Phenyl Diazirine Derivatives



Lewis acid	Yield of **2** (%)
$GaCl_3$	31
$FeCl_3$	47
SbF_5	80
$TiCl_4$	80

In the presence of $TiCl_4$, the reaction is endothermic, allowing large-scale preparation without external cooling. Thus, **2** can be obtained in multigram quantities. Further derivatization to give ester **4**, alcohol **5**,[8] and bromide **6**, as shown in Scheme 1, is straightforward. Compound **6** could also be obtained directly from **1** with $BrCH_2OCH_3$ in TfOH at 0 °C in a moderate yield.

Photoreactive peptides and proteins are powerful tools to identify interactions in protein networks. Photoreactive α-amino acids have been investigated because these analogs can be directly incorporated at specific positions in the polypeptide chain. For this reason, phenylalanine derivatives bearing a photophore, such as azide,[9] benzophenone,[10] or diazirine,[11] have found wide use; practical synthesis of the diazirine-bearing phenylalanine analog **3**, which has been successfully incorporated into peptides[12] and proteins,[13] is difficult though. The classical method via malonate gives a racemate of TmdPhe and requires a subsequent enzymatic resolution step to give the desired enantiomer.[11] The first asymmetric synthesis of TmdPhe was achieved in excellent yield[14] but required 1 equiv of a chiral Ni complex that is prepared by a rather complicated manner.[15] We adopted a cinchonidine-based asymmetric catalyst[16] for the alkylation of *tert*-butylglycinate benzophenone imine with bromide **6** in the presence of 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) as a base.[17] Gram quantities of TmdPhe **3** were easily obtained in excellent yield (86%) after the deprotection of **7** (Scheme 2). The optical purity of the Fmoc derivative **8** corresponded to an ee of 97%, as judged by HPLC analysis using a chiral support.

A photoreactive calmodulin-binding peptide (TmdPhe-CaMBP, **9**) was easily prepared using **8** via conventional solid-phase peptide synthesis. A biotin tag was incorporated at the N-terminus for detecting photolabeled proteins. The tryptophan residue in the original sequence of CaMBP[10] was replaced by TmdPhe to give the desired photoprobe **9** (MALDI-TOF MS *m*/*z* calcd for $[MH - 2N]^+$ 2330.51, found 2332.19). The site of modification was chosen on the basis of our earlier work in which a diazirine was successfully

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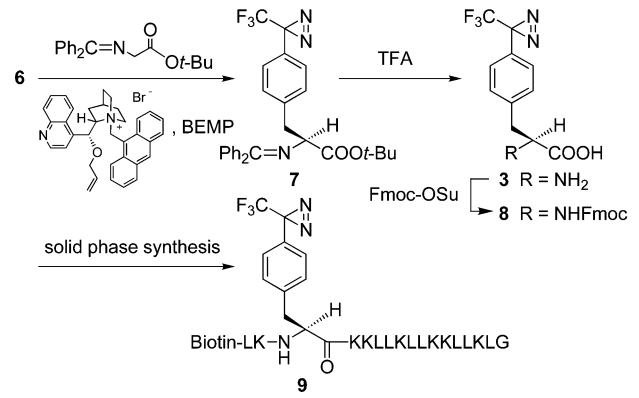
Scheme 2. Preparation of TmdPhe-CaMBP



introduced into the peptide via disulfide linkage by substituting the Trp^3 with cysteine.[18,19]

Figure 1 shows the photocross-linking of CaM with **9** visualized by chemiluminescence utilizing the interaction of HRP-conjugated avidin and biotin attached to CaMBP after blotting onto a PVDF membrane. Photolysis rapidly proceeded upon UV-A irradiation with 30 W/m^2 of 360 nm light at 0 °C and was complete in a few minutes (see Supporting Information). Labeled CaM was not detected in the absence of calcium (lane 2 in Figure 1a), as the benzophenone-tagged CaMBP was.[10,18] Moreover, when trifluoperazine was added to the solution as an inhibitor, the amount of labeled CaM decreased in response to the concentration of trifluoperazine (Figure 1b). These data indicate that the affinity of synthetic peptide **9** for CaM varies in a calcium-dependent manner.

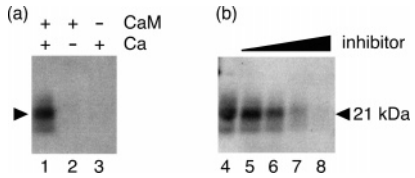


Figure 1. (a) Calcium dependence of the photolabeling of CaM with TmdPhe-CaMBP. Samples of 50 μL of Tris-HCl (50 mM, pH 7.4) containing CaM (5 μM), TmdPhe-CaMBP (**9**, 10 μM), $CaCl_2$ (10 mM), and NaCl (0.15 M) were irradiated at 0 °C for 2 min with a 30-W long-wavelength UV lamp after incubation at 37 °C for 10 min in the dark. Lane 1: in the presence of 10 mM $CaCl_2$. Lane 2: in the presence of 5 mM EGTA without $CaCl_2$. Lane 3: in the absence of CaM. (b) Competitive inhibition of photolabeling with trifluoperazine. Lane 4: same condition as Lane 1 in panel a. Lane 5: the concentration of trifluoperazine was 10 μM. Lane 6: 30 μM. Lane 7: 100 μM. Lane 8: 300 μM.

Although diazirine photophores have been recognized as useful photocross-linkers to probe the interfacial structure of protein−protein or protein−peptide complexes, the preparation of diazirine-based probes has not been convenient. As a consequence, progress in diazirine-based photoaffinity labeling has been limited. In this work, we dramatically improved the direct formylation of **1**, a practical diazirine source, to give compound **2** as a versatile diazirine unit for developing various elaborated diazirine analogs. The method enabled the practical synthesis of the useful photoreactive amino acid analog L-TmdPhe (**3**) on a gram scale suitable for standard solid-phase synthesis. The photoreactive peptide **9** containing this amino acid bound CaM and specifically labeled it in a calcium-dependent manner upon irradiation.

Photoreactive analogs of peptides, peptide-derived molecules, and proteins can provide structural information about transient interactions of proteins and facilitate tracking of signaling processes, transport/metabolism pathways, immune responses, and so on. Recently, this approach has received much attention in molecular biology for proteomic analysis owing to the success of in vitro[13,20] and in vivo[21] site-specific incorporation of a photoreactive moiety into polypeptides or proteins. Our method has the potential to make diazirine photophores useful building blocks for the preparation of diverse photoaffinity probes. It can therefore extend the potential of photoaffinity labeling as a sensitive means of rapidly elucidating protein structures and proteomic profiling.[22]

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Supporting Information Available: Experimental details and analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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